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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

9192.9USWO

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)

Unknown **09/254529**

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PRIORITY DATE CLAIMED

PCT/GB97/02859

October 17, 1997

October 17, 1996

TITLE OF INVENTION

RETROVIRAL VECTORS

APPLICANT(S) FOR DO/EO/US

Susan KINGSMAN et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
- ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
- ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
- ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
- ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
- ☒ An **UNSIGNED** oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
- ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: International Search Report, Form PCT/RO/101, Form PCT/ISA/220, Form PCT/IPEA/408, Response to written opinion dated November 9, 1998; Form PCT/IPEA/416, Information Disclosure Statement, PTO Form 1449, and copies of cited references (5)

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) Unknown	INTERNATIONAL APPLICATION NO. PCT/GB97/02859	ATTORNEY'S DOCKET NUMBER 9192.9USWO
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17. [X] The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)): Search Report has been prepared by the EPO or JPO..... \$840.00 International preliminary examination fee paid to U.S. Patent and Trademark Office (37 CFR 1.492(a)(1))..... \$670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))..... \$760.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(3)) paid to USPTO..... \$970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... \$96.00	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">CALCULATIONS</td> <td style="text-align: center;"><small>PTO USE ONLY</small></td> </tr> <tr> <td colspan="2" style="height: 150px;"></td> </tr> </table>	CALCULATIONS	<small>PTO USE ONLY</small>		
CALCULATIONS	<small>PTO USE ONLY</small>				

ENTER APPROPRIATE BASIC FEE AMOUNT =		\$840.00
Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	16	-20 = 0	X \$18.00	\$0.00	
Independent claims	1	-3 = 0	X \$78.00	\$0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ \$260.00	\$0.00
TOTAL OF ABOVE CALCULATIONS =				\$840.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$0.00	
SUBTOTAL =				\$840.00	
Processing fee of \$130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				+ \$0.00	
TOTAL NATIONAL FEE =				\$840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				+ \$0.00	
TOTAL FEES ENCLOSED =				\$840.00	

	Amount to be: refunded	\$
	charged	\$

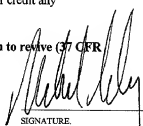
a. [X] Check(s) in the amount of \$840.00 to cover the above fees is enclosed.

b. [] Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
 A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
 overpayment to Deposit Account No. 13-2725.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
 Michael B. Lasky
 MERCHANT & GOULD
 3100 Northwest Center
 90 South Seventh Street
 Minneapolis, MN 55403


 SIGNATURE

 Michael B. Lasky
 NAME

 29,555
 REGISTRATION NUMBER

09/254529

300 Rec'd PCT/PTO 08 MAR 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Susan KINGSMAN et al. Docket No.: 9192.9USWO
Serial No.: UNKNOWN Filed: March 8, 1999
Int'l Appln No.: PCT/GB97/02859 Int'l Filing Date: October 17, 1997
Title: RETROVIRAL VECTORS

CERTIFICATE UNDER 37 CFR 1.10:

"Express Mail" mailing label number: EL176166025US

Date of Deposit: March 8, 1999

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

By: 

Name: Tina Just

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D. C. 20231

Dear Sir:

In connection with the above-identified application filed herewith, please
enter the following preliminary amendment:

IN THE ABSTRACT

Insert the attached Abstract page into the application as the last page
thereof.

IN THE SPECIFICATION

A courtesy copy of the present specification is enclosed herewith, but the
World Intellectual Property Office (WIPO) copy should be relied upon if it is already in
the U.S. Patent Office.

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IN THE CLAIMS

In claim 4, line 1, please delete "any one of claims 1 to 3" and insert --

claim 1--

In claim 5, line 1, please delete "any one of claims 1 to 4" and insert --

claim 1--

In claim 6, line 1, please delete "any one of claims 1 to 5" and insert --

claim 1--

In claim 7, line 1, please delete "any one of claims 1 to 6" and insert --

claim 1--

In claim 8, line 1, please delete "any one of claims 1 to 7" and insert --

claim 1--

In claim 9, line 1, please delete "any one of claims 1 to 8" and insert --

claim 1--

In claim 10, line 2, please delete "any one of claims 1 to 9 " and insert --

claim 1--

In claim 12, line 1, please delete "or claim 11"

In claim 13, line 2, please delete "any one of claims 10 to 12" and insert --

claim 10--

In claim 14, line 3, please delete "any one of claims 1 to 9" and insert --

claim 1--

In claim 15, line 1, please delete "any one of claims 1 to 9" and insert --

claim 1--

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REMARKS

A new abstract page is supplied to conform to that appearing on the publication page of the WIPO application, but the new Abstract is typed on a separate page as required by U.S. practice.

The above preliminary amendment is made to remove multiple dependencies from claims 4-10 and 12-15.

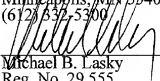
Applicant respectfully requests that the preliminary amendment described herein be entered into the record prior to examination and consideration of the above-identified application.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicant's primary attorney-of record, Michael B. Lasky (Reg. No. 29,555), at (612) 336-4634.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL,
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Dated: March 8, 1999

By: 
Michael B. Lasky
Reg. No. 29,555
MBL/kcd

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ABSTRACT

Retroviral vector particles having an RNA genome carrying sequences which provide in the DNA provirus at least one selected gene located within an intron in a transcription unit of the provirus, which transcription unit further comprises a polynucleotide response element responsive to a nucleus to cytoplasm transport factor such as HIV Rev. Expression of the selected genes is thus rendered Rev-dependent and so is dependent upon the presence of HIV.

0254529-080490

SMALL BUSINESS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

- a) ☐ the owner of the small business concern identified below:
b) ☐ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: OXFORD BIOMEDICA (UK) LIMITED
ADDRESS OF CONCERN: Medawar Centre
Robert Robinson Avenue
The Oxford Science Park
Oxford OX4 4GA
GREAT BRITAIN

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 C.F.R. 121.801-805, and reproduced in 37 C.F.R. 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled RETROVIRAL VECTORS by inventor(s) Susan Mary KINGSMAN and Alan John KINGSMAN described in

- a) ☐ the specification filed herewith.
b) ☐ provisional application serial no. __, filed __.
c) ☒ non-provisional application serial no. 09/254529, Int'l Filing Date: October 17, 1997.
d) ☐ patent no. __, issued __.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 C.F.R. 1.9(c) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or a nonprofit organization under 37 C.F.R. 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. 1.27)

NAME: _____
ADDRESS: _____
a) ☐ INDIVIDUAL b) ☐ SMALL BUSINESS CONCERN c) ☐ NONPROFIT ORGANIZATION

NAME: _____
ADDRESS: _____
a) ☐ INDIVIDUAL b) ☐ SMALL BUSINESS CONCERN c) ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereof, or any patent to which this verified statement is directed.

X NAME: PETER JOHN NOLAN
X TITLE: DIRECTOR OF OPERATIONS OXFORD BIOMEDICA (UK) LTD
X ADDRESS: MEDAWAR CENTRE, OXFORD SCIENCE PARK, OXFORD, UK
X SIGNATURE: *[Signature]* Date: 24 JUNE 1999

RETROVIRAL VECTORS

This invention relates to retroviral vector particles and to DNA constructs encoding RNA genomes for retroviral vectors. In particular it
5 relates to retroviral vectors for gene therapy for treatment or prevention of retrovirus infections, such as HIV.

Therapeutic molecules for use in HIV gene therapy include ribozymes, trans-dominant proteins, scFv molecules, antisense constructs and TAR and RRE decoys (reviewed in Yu *et al.* 1994). These molecules
10 are envisaged to act both as therapy against already infected cells and as protective 'intracellular immunisation' (Baltimore, 1988) in uninfected cells. In addition, the use of toxins (suicide genes) or immunological markers has also been proposed as a means of killing infected cells and so reducing the viral load in the patient.

For many of these molecules, it is desirable that expression can be regulated. This is clearly the case for suicide genes, but the constitutive expression of a therapeutic protein may also be undesirable in that it may cause some cellular toxicity or lead to a host immunological response. Furthermore, toxic side effects are also possible from the
15 expression of RNA molecules in cells, including the inappropriate induction of interferon responses. In the case of HIV, the viral LTR promoter itself has the characteristic of being an inducible promoter, directing low basal levels of transcription in the absence of the virally encoded Tat trans-activator protein (Arya *et al.* 1985). Tat-inducibility is a property of
20 sequences in the U3 region of the LTR and the TAR sequence in the R region (Berkhout and Jeang, 1992). Allowing the HIV LTR to direct expression of a therapeutic gene will therefore limit its expression to those cells infected by HIV. Several groups have already started exploring ways of using this property of the LTR and have demonstrated inducible
25 expression of genes upon HIV infection (Caruso *et al.* 1992, Brady *et al.*

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1994). Recently we have developed a novel vector system, the TIN system, that has clear advantages for HIV gene therapy (PCT/GB96/01230). This system comprises novel MLV based Tat-inducible (TIN) vectors, designed for the 5' positioning of a Tat induced therapeutic gene (TITG), and incorporating features to allow the efficient packaging, reverse transcription and integration of the vector genome by the MLV machinery. To facilitate the production of high levels of vector genomes in the producer cells (standard MLV packaging lines or our transient transfection system (Soneoka *et al.* 1995)), the packagable vector transcript is preferably driven from the CMV promoter rather than the natural vector MLV LTR. A clear advantage of this system is the achievement of HIV control of gene expression from a simple transcription unit which can be transduced by the standard MLV based vector technology. The TIN vector system was proposed to have additional advantages in that the low basal levels of transcription from the HIV LTR promoter would reduce problems of interference with any downstream constitutive promoters, facilitating stable expression of any constitutive gene from the vector in the transduced cells.

Where the therapeutic gene is a non natural protein there always exists the possibility of immune recognition and destruction of the cell expressing this protein. This is clearly undesirable if the aim is to create a reservoir of HIV resistant cells in the patient. It is therefore important to have basal levels of expression as close to zero as possible. The data using TIN vectors that we have previously described clearly demonstrate very low basal levels of expression but the levels were still detectable. Furthermore it is known that in some target cells additional cellular factors may be present that enhance the basal transcription from the HIV promoter in the absence of Tat. Indeed some transcription from the HIV LTR must occur to produce Tat itself. In the T cell population exposure to cytokines, antigens and various stress factors such as hypoxia

or UV irradiation are all known to stimulate transcription from the HIV LTR (Valerie *et al* 1988; Nabel & Baltimore 1987). Given that these types of stimuli are characteristic features of immune responses there therefore exists the possibility that basal expression from TIN vectors could be
5 elevated under certain conditions within an HIV infected patient. It is therefore desirable to provide additional controls on the basal levels of expression.

There is an additional regulatory circuit in HIV gene expression which involves the virally encoded Rev protein and its target
10 RNA sequence the Rev response element (RRE) (reviewed by Cullen 1995 and explored by Naldini *et al* 1996). HIV has two introns in its genome and regulates splicing to generate unspliced RNA genomes and two classes of sub-genomic mRNA. To ensure that some RNA remains unspliced and is exported to the cytoplasm for incorporation into new virus particles the
15 second intron contains a specific recognition sequence for a virally encoded protein called Rev. This RNA sequence is the Rev response element (RRE), for which a minimal functional sequence of 270 nucleotides has been identified (Huang *et al* 1991). Rev is a 13kD protein that specifically binds to RRE and activates the export of RRE-containing
20 RNA (Malim *et al* 1989b). It appears that the HIV introns are recognised by the cellular splice commitment factors; but the splicing process is inefficient which leads to entrapment of the RNAs within the nucleus. The Rev/RRE interaction bypasses this entrapment and exports the RNA to the cytoplasm. If splicing is made more efficient then the RNA export becomes
25 Rev independent (Chang and Sharp 1989; Hammariskjold *et al* 1994). If however gene expression is made more efficient then unspliced RNA can appear in the cytoplasm (e.g. D'Agostino *et al* 1992). In addition certain sequence elements referred to as cis inhibitory sequences (CRSs) might contribute to the Rev dependency of HIV by promoting nuclear entrapment
30 and/or RNA instability (e.g. Cochrane *et al* 1991). There is therefore an

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interaction between splicing efficiency, expression efficiency and CRSs that may influence Rev dependency. The interaction is not necessarily predictable or definable and may vary in different cell types (as discussed by Cullen 1995). The mechanistic details of Rev-mediated export have not yet been established but cellular export proteins are involved (e.g. Fritz *et al* 1995).

One potential approach to achieving additional regulation of a retroviral vector for HIV gene therapy would therefore be to incorporate RRE into the vector rendering expression dependent upon Rev. Such an approach may be obvious for vectors specifically based upon HIV itself as these will of necessity contain the Rev/RRE system, unless removed. For example Naldini *et al* (1996) use a fragment of HIV genome encompassing the normal major splice donor sequence, RRE and the normal splice acceptor sequence in the normal configuration in their transducing vector pHR'. RRE is therefore contained within the HIV env intron. This fragment is presumed and is likely, but not proven, to render the expression of the genome responsive to Rev. In the vector described in this paper the coding sequence lies outside of the RRE containing intron.

A non-lentiviral retroviral vector incorporating the Rev/RRE system has been described by Lisiewicz (US patent publication PB92-139336) whereby the RRE element is inserted into a retroviral vector or into the intron of a foreign gene contained within that vector. The constructions outlined make no reference to the presence of the splice donor sequence within the MLV vector, nor take into account any requirement for inefficient splicing to achieve Rev function, and there is no description of precisely where to insert the RRE. The disclosure therefore suggests that in constructing a retroviral vector whose expression is dependent upon Rev the nature and location of the RRE is not material and the nature and location of additional introns or splice sequences is not material.

We now show that a TIN vector can be converted into a Rev responsive vector by the insertion of RRE to create TRIN (Tat and Rev inducible) vectors. However, contrary to the finding in Lisiewicz, we demonstrate that simply inserting RRE into a TIN vector does not achieve a strict dependency on Rev and that there is therefore no significant advantage in this construction with respect to reducing basal gene expression beyond that obtained with TIN vectors. Moreover, we show that the inclusion of an extended sequence derived from the HIV env region which contains RRE and the 3' splice acceptor sequence from env produces a new vector pTRAC for which the basal transcription levels are undetectable in the absence of Tat and Rev. We propose that this undetectable expression is due to the combination of nuclear retention of the RNA via recognition of a hybrid MLV/HIV intron and the fact that any RNA that is spliced rather than exported via the Rev system will have the reporter/therapeutic gene removed during splicing.

The invention therefore provides in one aspect a retroviral vector particle comprising a packagable RNA genome capable of being inserted into a target cell genome when in the form of a DNA provirus, said RNA genome carrying sequences which provide in the DNA provirus at least one selected gene capable of being expressed in the target cell and located within an intron in a transcription unit of the provirus, which transcription unit further comprises a polynucleotide response element responsive to a nucleus to cytoplasm transport factor.

In another aspect, the invention provides a DNA construct encoding the packagable RNA genome for the retroviral vector particle described herein, under the control of a promoter. The selected gene or genes may be present in or absent from the DNA construct. If they are absent, the DNA construct has an insertion site e.g. a unique restriction enzyme site at which the selected gene or genes may be inserted, the site

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located within the intron such that it is flanked by a splice donor and a splice acceptor sequence.

In a further aspect, the invention provides a retroviral vector particle production system comprising a host cell transfected with a DNA construct as described herein, said system capable of producing retroviral vector particles as described herein. The host cell may be a packaging cell line or it may be a suitable host cell transfected with nucleic acid sequences, present e.g. on plasmids, encoding the structural elements of the retroviral particles. The transfected host cell is also referred to as a producer cell.

In yet another aspect, the invention provides a retroviral vector particle production system comprising a set of nucleic acid sequences encoding the components of a retroviral vector particle as described herein.

In a still further aspect, the invention provides the use of retroviral vector particles as described herein for gene therapy, and infected or transduced target cells resulting from such use.

The retroviral vector particle according to the invention therefore provides a means for inserting into a target cell a therapeutic gene whose expression in the target cell is dependent upon the presence of a factor which enables the transport of an RNA transcript containing the transcribed gene, into the cytoplasm.

The response element in the retroviral vector genome is chosen according to the specific conditions under which expression of the therapeutic gene is desired. If expression is to be HIV dependent, then a suitable response element is RRE or a functional equivalent which responds to Rev. A functional equivalent of RRE may be for example a portion of RRE or a mutated or otherwise manipulated version of RRE which retains the desired activity.

The transport factor may thus be HIV Rev, rendering expression of the therapeutic gene dependent upon the presence of HIV. The transport factor may alternatively be any factor, originating e.g. from other viruses or from host cells, which is analogous to Rev in that it enables transport to the cytoplasm via a specific interaction with the response element. Systems analogous to the Rev/RRE system are known, for example in other retroviruses. One such example is the rex/RxRE system in HTLV-1.

In order that RNA transcripts containing the transcribed therapeutic gene may be transported to the cytoplasm in the presence of the appropriate transport factor, the intron containing the therapeutic gene needs to be an inefficiently spliced intron. This may be achieved in a variety of ways. The preferred method described herein for achieving a Rev-dependent intron in an MLV-based vector is to use the MLV splice donor site and the HIV envelope gene 3' splice site. It is within the ability of those skilled in the art to devise other suitable splice site or intron sequence combinations for HIV Rev-dependency.

Preferably, the RRE or other response element is located within the intron, to ensure Rev- (or other transport factor) dependent expression. Conveniently, RRE and the 3' splice acceptor site may be provided in a sequence derived from the HIV env region.

Advantageously, the genome of the retroviral vector particle according to the invention will have its packaging site located within the intron containing the therapeutic gene. This means that RNA that is unspliced will be trapped in the nucleus and therefore unavailable for packaging, and RNA that is spliced will have the packaging site deleted and will also be unavailable for packaging. This feature contributes to the safety of the vector system, and is present in the preferred vector configuration described herein.

The retroviral vector particle may be MLV-based. MLV systems are so far the most widely used retroviral vector systems and have been used in human gene therapy applications. Other retroviruses may be used instead however, including other oncoretroviruses (the sub-group of retroviruses containing MLV), and lentiviruses (the sub-group of retroviruses containing HIV). Examples include ASLV, SNV and RSV all of which have been split into packaging and vector components for retroviral vector particle production systems. The retroviral vector particle according to the invention may be based on a genetically or otherwise (e.g. by specific choice of packaging cell system) altered version of a particular retrovirus.

That the vector particle according to the invention is "based on" a particular retrovirus means that the vector is derived from that particular retrovirus. The genome of the vector particle comprises components from that retrovirus as a backbone. The vector particle contains essential vector components compatible with the RNA genome, including reverse transcription and integration systems. Usually these will include gag and pol proteins derived from the particular retrovirus. Thus, the majority of the structural components of the vector particle will normally be derived from that retrovirus, although they may have been altered genetically or otherwise so as to provide desired useful properties. However, certain structural components and in particular the env proteins, may originate from a different virus. The vector host range and cell types infected or transduced can be altered by using different env genes in the vector particle production system to give the vector particle a different specificity.

Preferably, the retrovirus vector genome contains the minimum retroviral material necessary to function. This is important from a safety aspect; possible reconstruction of infectious virus particles must be avoided. In any case, the retroviral vector will be replication defective.

Also, by avoiding expression of unwanted virus proteins in the target cell, undesirable immune responses are reduced. Gag-pol and env are therefore supplied in trans in the vector particle production system.

However the retrovirus vector genome clearly needs to
5 contain the elements required for obtaining sufficiently high viral titres of vector particles from the producer cell, and for allowing it to integrate into the target cell genome. It will be evident that in order to function as a vector the retroviral vector particle according to the invention will need to have a reverse transcription system (compatible reverse transcription and
10 primer binding sites) and an integration system (compatible integrase and integration sites) allowing conversion to the provirus and integration of the double-stranded DNA into the host cell genome. Additionally, the vector genome will need to contain a packaging signal.

In a vector particle according to the invention which is
15 designed for use in gene therapy against a particular retrovirus, there is preferably also a control at the transcriptional level which requires the presence of that retrovirus in order to initiate or upregulate transcription of the therapeutic gene or genes. For anti-HIV gene therapy, the vector genome is preferably constructed such that in the DNA provirus the 5' LTR
20 comprises HIV sequences sufficient to render the therapeutic gene Tat-inducible, and the therapeutic gene is located between the LTRs, yet the vector is based on a simple retroviral vector such as MLV. The therapeutic gene is under the transcriptional control of the promoter in the 5' LTR but not otherwise operably linked to any other promoter from the vector
25 genome. This is the principle behind the TIN vectors discussed above.

In more detail, TIN vectors are constructed with the following points in mind. Tat acts on the TAR region of R, but also requires sequences in the U3 region to function properly. Certain sections of both U3 and R can be removed while still leaving an effective Tat-responsive
30 element. Deletions in HIV-2 U3 result in promoters with lower basal levels

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of transcription that still remain responsive to Tat (Brady *et al* 1990). Thus, the transcriptional control is preferably provided by an HIV Tat-inducible promoter comprising the functional portions of both U3 and R from HIV. Alternatively certain U3 and/or R sequences from other retroviruses, which are responsive to the HIV Tat protein, may be used, for example U3 from RSV linked to R from HIV. The TIN vector provides a means of preserving the Tat response within the context of a simple retroviral vector e.g. one based on MLV such that MLV packaging systems may be used.

The selected gene located in the intron of the vector genome according to the invention is preferably a therapeutic gene, that is it encodes a gene product which is active against infection or disease. Where the vector particle is for use in anti-HIV gene therapy, the product of the therapeutic gene will have an appropriate activity for that purpose. Therapeutic genes may encode for example an anti-sense RNA, a ribozyme, a transdominant negative mutant of a target protein, a toxin, a conditional toxin, an antigen that induces antibodies or helper T-cells or cytotoxic T-cells, a single chain antibody or a tumour suppressor protein.

Where two or more genes are present in the same transcription unit, there may be an internal ribosome entry site (IRES) e.g. from picornaviral RNA, to allow both genes to be translated from a single transcript. Retroviruses incorporating IRES sequences have been constructed by others.

It will be evident that the term "gene" is used loosely here, and includes any nucleic acid coding for the desired polypeptide. Usually, the genes delivered by the vector particle according to the invention will be cDNAs.

A further gene may also be present outside the intron containing the therapeutic gene and under the control of a separate promoter. This further gene may encode for example a selectable marker, or a further therapeutic agent which may be among the therapeutic agents

listed above. Expression of this gene may be constitutive; in the case of a selectable marker this may be useful for selecting successfully transfected packaging cells, or packaging cells which are producing particularly high titers of the retroviral vector. Alternatively or additionally, the selectable
5 marker may be useful for selecting cells which have been successfully infected with the retroviral vector and have the provirus integrated into their own genome.

One way of performing gene therapy is to extract cells from a patient, infect the extracted cells with a retroviral vector and reintroduce the
10 cells back into the patient. A selectable marker may be used to provide a means for enriching for infected or transduced cells or positively selecting for only those cells which have been infected or transduced, before reintroducing the cells into the patient. This procedure may increase the chances of success of the therapy. Selectable markers may be for
15 instance drug resistance genes, metabolic enzyme genes, or any other selectable markers known in the art.

However, it will be evident that for many gene therapy applications of retroviral vectors, selection for expression of a marker gene may not be possible or necessary. Indeed expression of a selection marker,
20 while convenient for *in vitro* studies, could be deleterious *in vivo* because of the inappropriate induction of cytotoxic T lymphocytes (CTLs) directed against the foreign marker protein. Also, it is possible that for *in vivo* applications, vectors without any internal promoters will be preferable. The presence of internal promoters can affect for example the transduction titres
25 obtainable from a packaging cell line and the stability of the integrated vector.

The DNA construct according to the invention which encodes the packagable RNA genome preferably comprises a promoter originating from a source other than the first or second retrovirus. Particularly
30 preferred are strong promoters such as the CMV promoter which give rise

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to a high level of expression of the vector RNA in the producer cell line.

The invention will now be further described with reference to the accompanying drawings in which:

Figure 1 shows a schematic outline of constructs according to the invention. The vector genome in the producer cell is shown (i). In the producer cell Rev is provided either by cotransfection or in a stable cell line by procedures known to those ordinarily skilled. The resulting vector derived RNA (ii) is unspliced and is exported to the cytoplasm by the Rev protein. In the target cell the vector genome is as in (i) but the only RNA that appears in the cytoplasm has the structure shown in (iii) because Rev is absent. Upon HIV infection when Rev is produced by the infecting virus then the retroviral vector derived RNA that reaches the cytoplasm will have the structure shown in (iv) i.e. identical to that in the producer cell (ii);

Figure 2 shows vectors used in the construction of a universal TIN vector (pTIN511);

Figure 3 shows construction of an RRE splice acceptor cassette (RAC);

Figure 4 shows construction of pTRAC;

Figure 5 shows construction of pTRAC-TG;

Figure 6 shows construction of pTRIN-TG; and

Figure 7 shows the prototype TIN vector pTIN414.

Figure 8 shows the principle of TIN vectors. The specific example of an MLV-based Tat-inducible vector is given. The packagable RNA genome comprises from the 5' end at least the functional part of the R region of HIV, all or a functional part of the MLV U5 region, a functional MLV primer binding site for first strand reverse transcription, a functional MLV packaging site, an insertion site for insertion of one or more therapeutic genes (into the DNA copy), a functional MLV primer binding site for second strand reverse transcription, a short (e.g. 10 - 100 nucleotides) sequence recognised by the MLV integration system, all or a

substantial part of the HIV U3 region, and an R region corresponding to the R region at the 5' end. Vector and proviral DNA are also shown.

Preparation of the constructs is described in detail in the Examples which follow.

5 This is the first description of the incorporation of an extended HIV-1 RRE element combined with an HIV-1 splice acceptor site into an MLV vector. It is the first description of a retroviral vector where the therapeutic gene is contained within an intron. The invention is particularly useful when combined with a TIN vector. That vector could be a single
10 transcription unit vector or could contain a second transcription unit as described for pTIN501. The retroviral vector could be based on any retrovirus including lenti-viruses. In the case of lentiviral vectors a vector has been described that contains the HIV-1 RRE and the major splice site but the therapeutic gene is not included within an intron and the Rev/RRE
15 system is not used to manipulate the expression of the therapeutic gene (Naldini *et al* 1996). The components of the intron in the present invention are preferably derived from MLV and HIV.

COMPONENTS OF MLV BASED TAT-INDUCIBLE (TIN) VECTORS

20 We have constructed a series of vectors that are packagable by standard MLV components, can be reverse transcribed and integrated by the MLV machinery following infection or transduction of a cell, and will allow Tat-inducible expression of a therapeutic gene from the transduced vector. The basic components of such a vector are as follows (Figure 8):

25
1. **Tat-inducibility.** The Tat-inducibility of the HIV LTR promoter is a property of sequences in the U3 region of the promoter and also the TAR element in R (Berkhout and Jeang, 1992). In addition, some other promoters, including the U3 regions from HIV-2 and RSV can substitute for
30 the HIV-1 U3 regions to allow Tat transactivation (Liu *et al.* 1994). In order

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for the U3 element to appear in the 5' LTR following reverse transcription, it must be present in the 3' LTR of the viral RNA. The vector therefore contains the HIV U3 and R sequences at the 5'LTR.

5 **2. Reverse transcription.** MLV RT initiates reverse transcription at the primer binding site (PBS). This initial (-) strand synthesis extends into U5 and R sequences, forming the first 'strong stop' DNA strand. The RNaseH moiety of RT then degrades the RNA in this hybrid, allowing the exposed DNA to hybridise with the homologous R region in the 3' LTR of the provirus. The homology between the 5' and 3' R regions enables the polymerase to switch strands and continue synthesis along the (-) strand from the 3'LTR. (+) strand DNA synthesis is primed by the selective retention of an RNA fragment at the polypurine tract after RNase degradation of the genomic RNA strand (reviewed in Katz and Skalka, 10 1994). The minimum requirements for MLV pol directed reverse transcription contained in the vector are therefore the PBS to initiate (-) strand DNA synthesis, the PPT to initiate (+) strand DNA synthesis and identical 5' and 3' R sequences to allow the first template switch. The requirement for identical R sequences is met by having HIV R sequences 15 in both 5' and 3' LTRs. In addition, as there is evidence to suggest that secondary structures in the 5' U5 region are also important for the initiation of reverse transcription (Cobrinck *et al.* 1991), we have kept the MLV U5 sequences in the 5'LTR. The 3' U5 sequences do not appear in the genomic RNA transcript; however, to ensure correct termination at the 3' 20 R/U5 border during genomic transcription, we will use the HIV U5 region in the 3' LTR.

3. **Integration.** The termini of the reverse-transcribed molecule contain short, sometimes imperfect, inverted repeats of 2-23 bp, which the 30 retroviral integrase recognises (reviewed in Katz and Skalka, 1994). For

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MLV, it has been demonstrated that only 9 bases at the end of a linear model substrate are sufficient for almost wild-type levels of integration in an in vitro integration assay system (Bushman and Craigie, 1990). These sequences are derived from the ends of the 3'U3 region and the 5'U5 region in the vector. This requirement is met by the vector containing 36 bases of MLV sequence at the 5' end of the 3' U3 and the whole of the MLV 5' U5 region.

4. Packaging components. Efficient packaging of a vector genome into a retroviral particle is dependent on a number of cis-acting sequences (reviewed in Linial and Miller, 1990). The most important sequence is the packaging signal, a highly structured region of RNA at the 5' region of the genome. In addition, other regions of the genome have been found to increase the efficiency of packaging, including sequences in gag p15. This region is included in standard MLV retroviral vectors, such as LXS_N (Miller and Rosman, 1989) and is also preserved in our constructs.

5. High titer retrovirus stocks following transient transfection. We have recently devised a system for the rapid production of high titer retroviral vectors (10^7 /ml) by transient transfection (Soneoka *et al.* 1995). A key feature of this system is that the powerful CMV promoter drives high level expression of the vector RNA in the producer cell line and is positioned so that the transcription start site of the vector RNA is exactly the same as the normal LTR-directed start site. To achieve this, we have placed the CMV promoter up to its transcriptional start site adjacent to the start of the HIV R region in the 5' LTR. This principle could be applied to other heterologous promoters, as long as the integrity of the retroviral transcription unit is maintained.

This system has been described for hybrid MLV-HIV vectors, but the same principle can be applied to other combinations of

retroviruses, where one retrovirus is donating the cis-acting sequences required by its own packaging components provided in trans (e.g. SNV, RSV, ASLV etc.) and the other retrovirus is used because of the property of conditional expression of its LTR promoter. Further examples of such retroviral promoters are the HTLV-1 promoter (dependent on Tax protein) and the steroid-hormone inducible MMTV LTR (reviewed in Majors, 1990). The use of HTLV-1 LTR, for example, to direct expression of a suicide gene could find applications as a treatment for adult T-cell leukemia.

10 **ADVANTAGES OF THE VECTORS DESCRIBED HEREIN**

1. Reduced basal levels of expression of therapeutic genes leading to reduced toxicity and immune recognition of target cells.
2. Stricter dependency upon HIV infection to activate gene expression
- 15 3. Reduced availability of RNA carrying the packaging site leading to lower risk of inadvertent packaging of the RNA in the target cell.

EXAMPLES

20 **I. Construction of a universal TIN vector (pTIN511) (Figure 2)**

The starting molecule is pTIN500 (described in PCT/GB96/01230). This was derived from pTIN414 described in PCT/GB96/01230 (and described herein in detail below and illustrated in Figure 7). PTin414 is the TIN vector equivalent of retroviral vector PHIT111 (Soneoka *et al* 1995), a derivative of LZSN (Adam *et al* 1991). Subsequent TIN vectors were derived from pTIN414 by replacement of internal sequences between unique SpeI and NheI sites. The SpeI site is located within the non-translated gag coding region upstream of the lacZ gene and the Nhe I site is in the 3' U3 region at the junction of the MLV and HIV-1 sequences. Plasmid pTIN500 contains the SpeI - NheI internal

30

fragment from pBABEpuro. The SV-Puro cassette is deleted by digestion with *AccI* and religation. This produces pTIN510. A poly-linker is inserted into the unique *EcoRI* site in pTIN510.

5 Polylinker sequence (SEQ ID NO: 1)

EcoRI Sall XhoI BglII EcoRI
5'AATTCGTCGACCTCGAGATCCG;
GCAGCTGGAGCTCTAGGCAATT 5'

10

This creates pTIN511 which has unique Sall, XhoI and BglII sites for the insertion of additional sequences.

The removal of the SV-Puro cassette is not critical for the current invention but serves to simplify the structure of the vector. There may be situations, obvious to one skilled in the area, when the retention of this or any additional cassette might be desirable.

15

Detailed construction of TIN414.

The co-ordinates of the sequences derived from the CMV promoter, the MLV vector LZSN (Adam *et al.* 1991) and the HIV-1 proviral clone WI3 (Kim *et al.* 1989) are indicated. The molecule was created using standard recombinant DNA techniques and in addition, recombinant PCR to create exact junctions between the different parts of the molecule (Higuchi 1990). Specifically

25

(i) Construction of the 5' CMV driven LTR

Plasmid pPE611 (Braddock *et al.* 1989) contains the human CMV promoter (from -521 to +1) joined exactly to the start of the HIV-1 R region (co-ordinates +1 to +80). An *XbaI* - *BamHI* fragment from this plasmid was ligated into the cloning vector pBluescript (Stratagene) to give

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plasmid pRV404. A PCR amplification was performed using plasmid pLNSX as the template, using primers 5'-
gcgagctagcttgaatcggtctcgctgttcctgg-3'
and 5'ggcgctagcgttcagaactcgtcagttccaccac-3'. The PCR product so
5 generated was digested with NheI and ligated into pRV404 at its NheI site
to give plasmid pRV405. Two oligonucleotides of sequence 5'-
ttaagcctcaataaagctgccttgagtgcttcac-3' and 5'-
cgatgaagcactcaaggcaagccttattgagc-3' were annealed together to create
a short duplex containing single stranded regions at either end
10 corresponding to the overhangs present on AflII and BstBI restriction
fragments. This molecule was ligated into plasmid pRV405 cut with AflII
and BstBI, to give plasmid pRV406.

(ii) Construction of the 3' LTR

15 A HindIII - XbaI fragment from pLNSX was ligated into the
cloning vector pSP72 to give plasmid pRV400. Plasmid pBX+ contains a
BamHI - XbaI fragment from WI3 containing the whole 3' LTR from the
HIV-1 genome. PCR amplification was performed on pBX+ using primers
5'-ccgcgctagcgatattccttgatctgtgatctaccac-3'
20 and 5'gcgagggtaccgtcgactgctagagatttccactgac-3'. The PCR product
was digested with KpnI and NheI and ligated into plasmid pRV400
digested with KpnI and NheI, to give plasmid pRV401. The ClaI - KpnI
fragment of pRV401 was ligated into pBluescript digested with ClaI and
KpnI, to give plasmid pRV408.

(iii) Addition of internal sequences.

25 A SacII - SpeI fragment from pRV406 was ligated into
plasmid pRV408 to create plasmid pRV412. The SpeI - NheI fragment from
pRV412 was replaced with the SpeI - NheI fragment from LZSN to give
30 vector pTIN414 (Figure 7).

2. Construction of the RRE splice acceptor cassette (RAC) Figure 3.

The starting molecule is pWI3 (Kim *et al* 1989). This contains a full length proviral clone of isolate HIV-1 IIIb clone HXB2 (Ratner *et al* 1985; Genbank accession no.K03455). Coordinates refer to nucleotide positions in the proviral DNA starting with the first nucleotide of the 5' LTR as 1 and are given in brackets following the restriction site. To facilitate cloning steps a subclone is created that contains only the envelope region of HIV-1. To achieve this a Sall (5785) to BamHI (8473) fragment from WI3 is inserted into the polylinker of the cloning vector pSP46 (Promega) to produce plasmid pPE531. The RRE splice acceptor region (RAC) is further subcloned as a BgIII (7620) to EcoRI (site in the pSP46 polylinker) into the polylinker site of pSP71 to create pRAC.

3. Construction of pTRAC (Figure 4)

The BgIII(7620) to BamHI (8473) fragment is inserted into the unique BgIII site in pTIN511. In the correct orientation the upstream BgIII site is preserved and the downstream site is a hybrid BgIII/BamHI site which is non-functional for either enzyme. pTRAC therefore retains three unique cloning sites upstream of the RAC .

4. Construction of pTRAC-TG (Figure 5)

A coding sequence of choice is inserted into one of the unique restriction sites in pTRAC. This sequence is a therapeutic gene or a reporter gene. The sequence is prepared with appropriate restriction at the termini and has an ATG codon for translation initiation. In the present example the RevM10 sequence is amplified from plasmid pM10 (Malim *et al* 1989a) using PCR primers incorporating flanking BgIII (upstream) and BamHI (downstream sites).

Primer sequences:

GGCAGATCTATGGCAGGAAGAAGCGG - 3' (SEQ ID NO: 2)

GGCGGATCCTTCTTTAGTTCCTGACTCC - 3' (SEQ ID NO: 3)

5

The amplified product is digested with BglI and BamHI and the product is ligated into the unique BglII site of pTRAC. The vector genome is renamed according to the therapeutic gene in this example pTRAC-TG becomes pTRAC-RevM10.

10

Insertion of the gene in the correct orientation preserves the upstream BglII site but the downstream site is destroyed by the formation of a BglII/BamHI hybrid site.

5. Construction of pTRIN and pTRIN-TG (Figure 6)

15

To construct an RRE cassette, a 359 base pair fragment encompassing the minimal fully functional RRE is amplified from pRAC using primers that locate with respect to the HIV proviral sequence coordinates at nucleotide 7705 and 8067 (or from pPE351 sequences from 7707 to 8066). The upstream primer adds an EcoRI site followed by a

20

BamHI site and the downstream primer adds an EcoRI site.

Primer 1 (SEQ ID NO: 4) (lower case is HIV-1 sequence 7705 to 7725)

5'CCGCGAATTCGGATCCaggagtagcaccaccaaggc

Primer 2 (SEQ ID NO: 5) (lower case is HIV-1 sequence from 8067 to 8047)

25

5'CCGCGAATTctccaactagcattccaaggc

The amplified product is digested with EcoRI and is ligated into the EcoRI site of pTIN510 to produce pTRIN. This now has a unique BamHI site for the insertion of any additional sequences.

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A therapeutic gene or reporter gene is inserted into the unique BamHI site of pTRIN. In the present example the RevM10 cassette described above is inserted. The vector is renamed according to the therapeutic gene in this example pTRIN-TG becomes pTRIN-RevM10.

5

6. Analysis of pTRAC-TG and pTRIN-TG

Retroviral vector stocks are produced either by transient transfection of 293T cells according to methods of Soneoka *et al* 1995 or by the creation of producer cell lines by standard methods e.g. (Cosset *et al* 1995). MLV packaging components are provided in trans on two plasmid components - a gag-pol expression plasmid (pHIT60, an MLV gag-pol expression plasmid described in Soneoka *et al* 1995) and an amphotropic envelope expression plasmid (pHIT456 derived from plasmid SV-A-MLV-env described in Page *et al* 1990 and essentially the same as the ecotropic expression construct pHIT123 described in Soneoka *et al* 1995). Rev is provided in trans from a CMV rev expression plasmid. The following vector genomes are used:-

- a) standard MLV vector (pHITIII; Soneoka *et al* 1995)
- b) pTRIN-RevM10
- 20 c) pTRAC-RevM10
- d) pTRIN-lacZ
- e) pTRAC-lacZ
- f) pTIN414 (as described in PCT/GB96/01230) and shown in Figure 7.

25

The following additional plasmids are used in the analysis:-

- g) pTAT (A CMV Tat expression plasmid as described by Braddock *et al* 1989)
- h) pHCMVsrev (A Rev expression plasmid as described by Benko *et al* 1990).

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Virus stocks are used to infect HeLa-CD4 cells and human U937 cells. In some cases repeat transfections are done as described by Cannon *et al* (1996). In this procedure the target cells are exposed to fresh retroviral vector stocks every 48 hours. Forty-eight hours after the final transduction the transduced cell populations are infected with HIV-1. After 48 and 72 hours cells are harvested and protein extracts are prepared by standard procedures and assayed for the expression of the marker or therapeutic gene. In addition viral spread through the culture is assessed by determining reverse transcriptase activity in culture supernatants every three days.

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CLAIMS

- 5 1. A retroviral vector particle comprising a packagable RNA genome capable of being inserted into a target cell genome when in the form of a DNA provirus, said RNA genome carrying sequences which provide in the DNA provirus at least one selected gene capable of being expressed in the target cell and located within an intron in a transcription
- 10 unit of the provirus, which transcription unit further comprises a polynucleotide response element responsive to a nucleus to cytoplasm transport factor.
2. The retroviral vector particle according to claim 1, wherein the polynucleotide response element is responsive to a transactivating
- 15 retroviral nucleus to cytoplasm transport factor.
3. The retroviral vector particle according to claim 2, wherein the polynucleotide response element is responsive to HIV Rev or a functional equivalent thereof.
4. The retroviral vector particle according to any one of claims 1
- 20 to 3, wherein the polynucleotide response element is the Rev response element (RRE) or a function equivalent thereof.
5. The retroviral vector particle according to any one of claims 1 to 4, wherein a selected gene is a therapeutic gene.
6. The retroviral vector particle according to any one of claims 1
- 25 to 5, based on an oncoretrovirus.
7. The retroviral vector particle according to any one of claims 1 to 6, based on murine leukemia virus (MLV).
8. The retroviral vector particle according to any one of claims 1 to 7, wherein the 5' long terminal repeat (LTR) of the provirus comprises
- 30 HIV U5 and R regions or functional portions thereof having Tat inducible

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promoter activity, in place of the 5' LTR promoter function of the retrovirus on which the vector particle is based.

9. The retroviral vector particle according to any one of claims 1 to 8, wherein the packaging signal is contained within the intron in which

the selected gene is located.

10. A DNA construct encoding the packagable RNA genome for the retroviral vector particle according to any one of claims 1 to 9, operably linked to a promoter.

11. The DNA construct according to claim 10, wherein the promoter is a strong promoter such as the CMV promoter.

12. The DNA construct according to claim 10 or claim 11, wherein the selected gene is absent and the construct has an insertion site within the intron at which the selected gene or genes may be inserted.

13. A retroviral vector particle production system comprising a host cell transfected with the DNA construct according to any one of claims 10 to 12, said system capable of producing retroviral vector particles according to any one of claims 1 to 8.

14. A retroviral vector particle production system comprising a set of nucleic acid sequences encoding the components of a retroviral vector particle according to any one of claims 1 to 9.

15. The use of a retroviral vector according to any one of claims 1 to 9 for gene therapy for infection or transduction of a target cell.

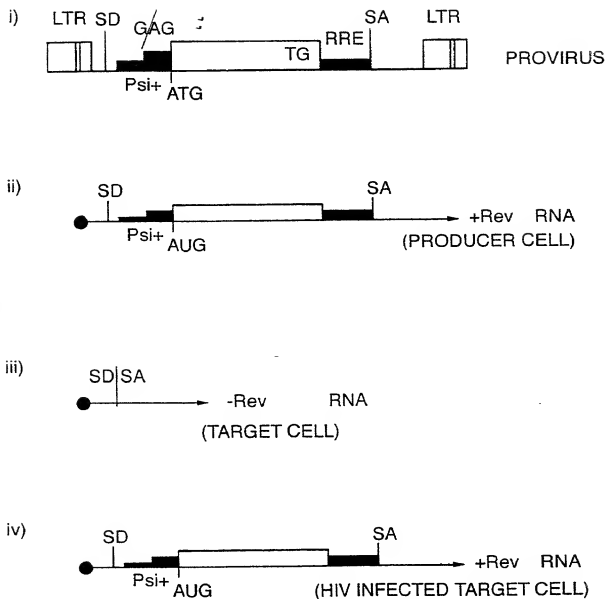
16. Target cells resulting from the method according to claim 15.

Patent application number **ELI7616602545**
 Date of filing **March 8, 1999**
 The following information is being reported
 to the United States Patent Office (USPTO) for
 filing in accordance with 37 CFR 1.10 on the
 date indicated above and to be addressed to the Com-
 missioner of Patents and Trademarks, Washington, D.C. 20231
Tim Just
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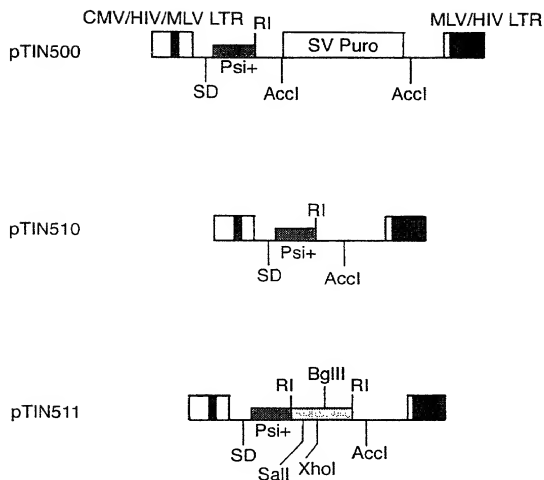
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Fig.1.



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Fig.2.



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Fig.3.

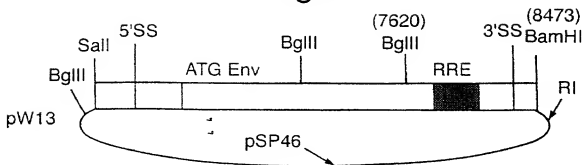


Fig.4.

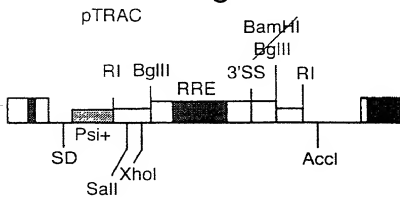
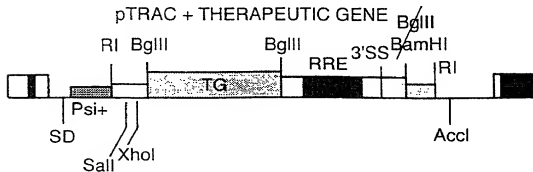


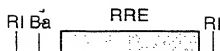
Fig.5.



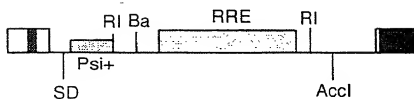
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Fig.6.

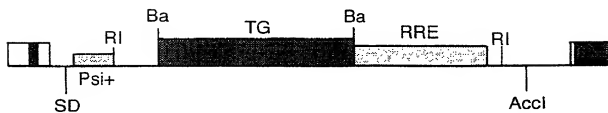
RRE CASSETTE



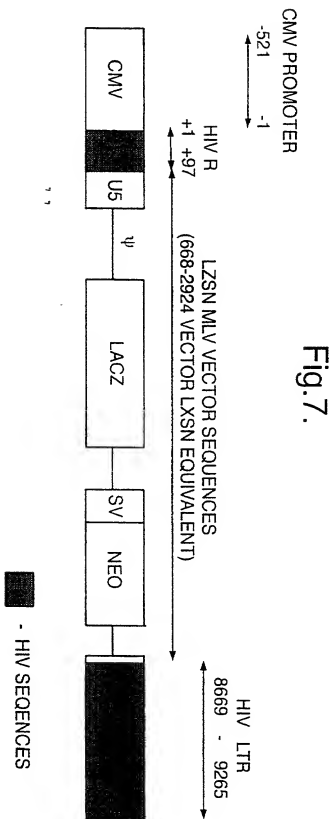
pTRIN



pTRIN-TG

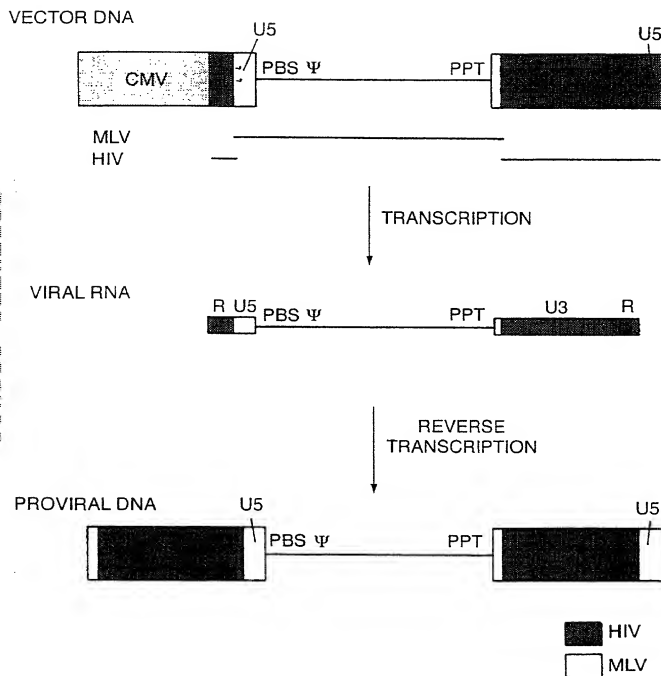


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Fig.8.



MERCHANT, GOULD, SMITH, EDELL, WELTER & SCHMIDT

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **RETROVIRAL VECTORS**

The specification of which

- a. ☐ is attached hereto
 b. ☒ was filed on _____ as application serial no. _____ and was amended on _____ (if applicable) (in the case of a PCT-filed application) described and claimed in international no. PCT/GB97/02859 filed 17 October 1997 and as amended on _____ (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

- a. ☐ no such applications have been filed.
 b. ☒ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
Great Britain	9621679.1	17 October 1996	
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

Albrecht, John W.	Reg. No. <u>40,481</u>	Lacy, Paul E.	Reg. No. <u>38,946</u>
Anderson, Gregg I.	Reg. No. <u>28,828</u>	Larson, James A.	Reg. No. <u>40,443</u>
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I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Merchant, Gould, Smith, Edell, Welter & Schmidt to the contrary.

Please direct all correspondence in this case to Merchant, Gould, Smith, Edell, Welter & Schmidt at the address indicated below:

**Merchant, Gould, Smith, Edell,
Welter & Schmidt**
3100 Norwest Center
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Minneapolis, MN 55402-4131

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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